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Infection With GB Virus C/Hepatitis G Virus Among Blood Donors and Hemophiliacs in Martinique, a Caribbean Island

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GB virus C/hepatitis G virus (GBV-C/HGV) RNA was detected by reverse transcription-polymerase chain reaction with primers derived from the nonstructural region 3 (NS3) in 9 (4.1%) of 221 blood donors and 2 of 20 (10%) hemophilia patients in Martinique, French West Indies. Anti-E2 antibodies were found in sera from 33 (14.9%) of the blood donors and 5 (25%) of the hemophiliacs. None of the subjects was positive for both GBV-C/HGV RNA and anti-E2. Among the 20 hemophiliacs, 12 (60%) had anti-HCV antibodies and 7 (35%) were positive for HCV RNA by PCR. All patients positive for HCV markers belonged to the group of 13 patients exposed previously to blood factor concentrates that were not activated virally. Nucleotide sequences of the 5′-untranslated region (5′UTR) of the GBV-C/HGV genome were obtained for the 10 NS3 PCR positive samples. Phylogenetic comparison of these isolates with reference isolates published previously showed a strong homology with European and American GBV-C/HGV strains, 8 isolates belonging to the genotype 2a and 1 isolate to the type 2b. The isolate from 1 blood donor was identified as subtype 1a , indicating the presence of West African type strains. ***J. Med. Virol. 59: 160–163, 1999.***

KEY WORDS: GBV-C/HGV; genotypes; hemophilia; blood donors; Caribbean

INTRODUCTION

GB virus C (GBV-C) and hepatitis G virus (HGV) are two isolates of the same new virus, belonging to the Flaviviridae family, with a similar genetic structure to hepatitis C virus (HCV) but with a sequence divergence too wide to be considered a genotype of HCV [Simons et al., 1995; Linnen et al., 1996].

The role of GBV-C/HGV in non-A-E hepatitis is not established, and this virus has not been associated definitely with any disease [Alter et al., 1996]. However, high rates of GBV-C/HGV infection in blood donor populations have been reported in various countries [Karayiannis et al., 1997; Ross et al., 1998]. GBV-C/HGV transmission by transfusion has been demonstrated with frequent viral persistence for several months or years in blood recipients [Linnen et al., 1996]. This prompted us to investigate the prevalence of this virus among volunteer blood donors and hemophiliacs in the West Indies island of Martinique as, to our knowledge, there are no data on GBV-C/HGV in Caribbean populations. Exposure to GBV-C/HGV was assessed by using reverse transcription-polymerase chain reaction (RT-PCR) in the NS3 region of the viral genome and by enzyme linked immunosorbent assay (ELISA) for antibodies to GBV-C envelope protein E2. In addition, we sequenced the 5′UTR of the detected isolates in order to determine the GBV-C/HGV genotypes present in this geographical area. The prevalence of hepatitis C (HCV) and hepatitis B (HBV) viruses markers was also evaluated.

MATERIALS AND METHODS

Subjects

Blood samples were obtained from 221 blood donors and 20 patients with hemophilia. The samples were collected between February and May 1996. The donors were unpaid and they were selected according to the French laws and guidelines: HIV1/2, HTLV1/2, HBV, and HCV serological markers were screened routinely and risk factors for these infections were ascertained before donation. Serum was separated from whole blood within 3 hours after collection and aliquots were stored at −80°C until further testing.

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Antibody Test to E2 Protein of GBV-C/HGV

IgG antibodies to GBV-C/HGV envelope protein E2 were detected using an ELISA (Boehringer Mannheim; Penzberg, Germany) as recommended by the manufacturer. Statistical analysis on the age of the positive and negative blood donors was carried out with Student's t-test.

Detection of GBV-C/HGV RNA

RNA was extracted from 100 μl of serum using the HCV Specimen Preparation (Roche Diagnostic Systems, Basel, Switzerland) according to the manufacturer's protocol. RT was undertaken by random priming using hexamers and Moloney murine leukemia virus reverse transcriptase (Life Technology, Grand Island, NY). Using consensus-degenerated primers gbvc-a1 (5′-ACNACNAGGTCNCCRTCYTTGATGAT-3′) and gbvc-s1 (5′-GGNRMKRTYCCYTTTTATGGGCATGG-3′) derived from the NS3 region of the GBV-C genome [Leary et al., 1996], 1 μl of the cDNA was amplified for 50 cycles. Each cycle entailed denaturing for 30 seconds at 94°C, annealing for 30 seconds at 55°C, and extension for 30 seconds at 72°C. Amplified products were detected by 2 % agarose gel electrophoresis and ethidium bromide staining. The expected size of the PCR product was 188 bp. This procedure was validated by participation in a French multicenter quality control group (Groupe Français d'Etude Moléculaire des Hépatites, GEMHEP).

Determination of GBV-C/HGV Genotype

Reverse transcription, amplification and direct sequençing of the amplified products from sera were carried out using the current version of the TrueGene™ High Resolution Subtyping GeneKit with the CLIP™ technology on the Microgene Clipper™ DNA Sequencer. Using this method a double-stranded sequence of 210 bp of the 5′ noncoding region of GBV-C, ranging from nucleotide 120 to 330 of the prototype HGV sequence PNF2161 (accession number U44402) can be obtained.

Sequence alignments were undertaken using the Clustal method of the Megalin programme of Lasergene Software. Phylogenetic analysis was undertaken using the Neighbor-joining method. Previously reported sequences were included in the alignment and subsequent phylogenetic analysis to determine the GBV-C/HGV genotype [Muerhoff et al., 1996].

HCV and HBV Serologic Assays

In all study samples, detection of serum HCV antibodies was carried out using ELISA tests (Monolisa anti-HCV, Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France and/or Ortho HCV 3.0, Chiron, Emeryville, CA) and validated by a recombinant immunoblot assay (RIBA 3.0, Ortho). Screening for HBsAg was performed by ELISA and confirmed by neutralisation (Monolisa, Sanofi Diagnostics Pasteur). Anti-HBc were detected by ELISA and positive samples were retested using an IMx Microparticle Enzyme Immunoassay (Abbott Laboratories, North Chicago, IL).

TABLE I. Epidemiological Features of GBV-C/HGV RNA or Anti-E2 Positive and GBV-C/HGV RNA and Anti-E2 Negative Blood Donors

	GBV-C/HGV RNA positive	GBV-C/HGV anti-E2 positive	GBV-C/HGV negative
Number of donors	9	33	179
Mean age, year (SD)	39 (9)	41 (8)	35 (11)
Sex ratio	0.7	0.4	0.7

Detection of HCV RNA

Serum samples from the hemophiliac patients were assessed for HCV RNA using an HCV kit (Amplicor, Roche Diagnostic Systems, Basel, Switzerland).

RESULTS

Prevalence Of GBV-C/HGV Infection Among Blood Donors

Of 221 serum samples from blood donors, 9 (4.1%) had GBV-C/HGV RNA detected by RT-PCR using the NS3-derived primers and 33 (14.9%) had anti-E2 antibodies present. No individuals had both GBV-C/HGV RNA and anti-E2 antibodies. The epidemiological characteristics of the GBV-C/HGV positive and negative blood donors are shown in Table I. GBV-C/HGV anti-E2 positive blood donors were significantly older than the GBV-C/HGV negative donors ($P < 0.005$).

HCV and HBV Prevalence Among Blood Donors

Prevalences of HCV antibodies, Hbs antigen and anti-HBc antibodies among the blood donor population of Martinique during the study period were 0.05% (4/7970), 0.35% (28/7970) ,and 4% (321/ 7970), respectively. Of the 221 blood donors studied for GBV-C/HGV infection, none had HCV antibodies or HbsAg and 11 had anti-HBc antibodies. Of the 11 anti-HBc antibodies positive donors, 1 was positive for GBV-C/HGV RNA and 2 had anti-E2 antibodies.

Prevalence Of GBV-C/HGV Infection Among Hemophiliacs

Two (10%) of the 20 hemophiliacs were positive for GBV/HGV RNA and 5 (25%) had anti-E2 antibodies. No patient carried both GBV-C/HGV RNA and anti-E2 antibodies. Among the 13 haemophiliacs with past exposure to noninactivated blood factor concentrates, 2 (15.4%) were positive for GBV-C/HGV RNA and 4 (30,8%) had anti-E2 antibodies; 1 of the 7 patients treated with only virus-inactivated concentrates carried anti-E2 antibodies.

HCV and HBV Status of the Hemophiliacs

Among the group of hemophiliacs, the prevalence of HCV infection was 60% (12/20) with 35% (7/20) viraemic patients. All but one (92.3%) of the 13 hemophiliacs

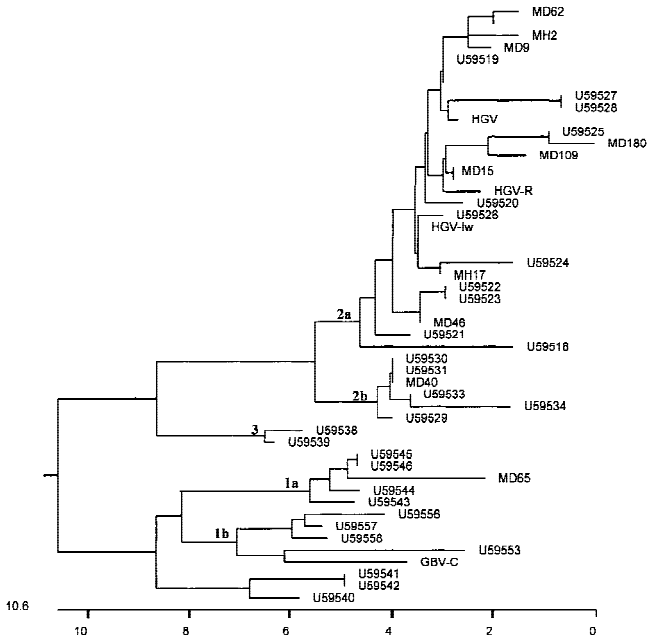

Fig. 1. Phylogenetic tree constructed from comparison of sequence in 5′-untranslated region of GBV-C and HGV using Clustal alignment and Neighbor-joining method from the Megalin programme (Lasergene software). The isolates examined in this study are indicated by the "MD" preceding the blood donor number and the "MH" preceding the hemophilia patient number. GBV-C is the GBV-C prototype [Simons et al., 1995]. HGV and HGV-R are respectively the isolates PNF2161 and R10291 reported by Linnen et al. (1996). HGV-Iw refer to a Japanese complete genome sequence [Shao and al., 1996]. The 5′-UTR sequences derived from strains registered between GenBank accession numbers U59518 and U59558 are included [Muerhoff et al., 1996].

exposed to non-virus-inactivated concentrates had anti-HCV antibodies and 7 (53.8%) of them, including the 2 patients with GBV-C/HGV RNA, were positive for HCV RNA. No individual with prior treatment by only noninactivated concentrates was positive for anti-HCV antibodies or HCV RNA. Of the 20 haemophiliacs tested, 9 (45%) had anti-HBc antibodies, one carrying GBV-C/HGV RNA and two anti-E2 antibodies. All the patients were negative for HBsAg.

GBV-C/HGV Genotype

Sequences were obtained from the 5′UTR region for the 8 NS3 PCR positive samples from blood donors and the 2 isolates from hemophilia patients. Alignment of these sequences with representative GBV-C/HGV 5′ end sequences reported earlier [Muerhoff et al., 1996], followed by phylogenetic analysis, resulted in the tree illustrated in Figure 1. Eight isolates, including the two strains from hemophilia patients, clustered within the GBV-C/HGV type 2a, and 1 isolate fell into the type 2b branch, genotypes that are usual in North America and Europe. One isolate, from a blood donor, was a strain of type 1a, which is common in Africa. On the nucleotide level, The 5′UTR sequences of type 2 isolates showed more than 95% of homology with each other and 85% homology with type 1a isolate.

DISCUSSION

This study has shown that the prevalence of GBV-C/HGV RNA and anti-E2 antibodies observed in hemophiliacs and blood donor populations in the French Caribbean island of Martinique, is high; 35% of the hemophiliacs and 19% of the volunteer blood donors have been exposed to the GBV-C/HGV virus. Among the hemophiliacs, the rates of GBV-C/HGV persistent infection and of anti-E2 recovery marker detection are similar to those reported in other countries [Linnen et al., 1996; Jarvis et al., 1996; Gerolami et al., 1997]. In patients with past exposure to noninactivated factor concentrates, the HCV infection markers are more frequent than the GBV-C/HGV infection markers. This suggests that HCV is more efficiently transmitted via noninactivated concentrates than GBV-C/HGV. Another possibility is that recovery from GBV-C/HGV infection is more frequent than recovery from HCV infection and that GBV-C/HGV anti-E2 antibodies disappear more rapidly than HCV serological markers.

The frequency of GBV-C/HGV infection observed in this study of blood donors is higher than the rates of 4–5.5% of blood donors with prior contact with GBV-C/HGV obtained in the USA [Dille et al., 1997; Gutierrez et al., 1997], lower than the data reported in Brazil or Africa for GBV-C/HGV RNA (9% and 14%) and anti-E2 (19.5% and 20.3%) [Dawson et al., 1996; Bassit et al., 1997; Ross et al., 1998], and slightly higher but consistent with the prevalence published in Europe [Jarvis et al., 1996; Prati et al., 1997; Tacke et al., 1997; Ross et al., 1998] and in continental France [Loiseau et al., 1997]. The prevalence of GBV-C/HGV in Martinique blood donors is much higher than the prevalence of HCV, as reported elsewhere. Therefore the existence of other major routes for GBV-C/HGV transmission is suspected. GBV-C/HGV sequences are present in the saliva and in the semen of infected individuals [Chen et al., 1997; Semprini et al., 1998]. The possibility of sexual or vertical transmission of GBV-C/HGV has been reported [Kao et al., 1997; Scallan et al., 1997; Feucht et al., 1996; Zanetti et al., 1998], but little is known about the rates of transmission. However, the comparison of GBV-C/HGV, HCV and HBV prevalence between French West Indies and Continental France suggests that for GBV-C/HGV, as for HCV, the sexual route of transmission is not as efficient as it is for HBV. Indeed, the frequency of HBV markers in the Martinique blood donor population is 5- to 10-fold higher than in France [Saura et al., 1997]. This is explained by the high rates of heterosexual transmission in the Caribbean and subsequently perinatal transmission of HBV in these populations. On the contrary, for GBV-C/HGV and HCV, the prevalences of infection in Martinique and Continental France are similar.

To determine the phylogenetic relation of GBV-C/HGV isolates from Martinique with isolates reported elsewhere, we analysed the homology of nucleotide sequences of a 210bp 5′end region. This method demonstrated variations among the 10 isolates of our study,

confirming the absence of cross-contamination. Phylogenetic analysis of these sequences with previously reported 5′UTR sequences [Muerhoff et al., 1997] showed the predominance of type 2a isolates, indicating that GBV-C/HGV strains in French West Indies are closely related to European/North American strains. The genetic homology among these type 2 isolates is high, suggesting that the GBV-C/HGV population present to date in the island of Martinique is rather homogeneous. However, the finding of a type 1a strain indicates that the West African type has also been introduced in this part of the world.

Additional studies are needed to understand the routes of transmission of GBV-C/HGV and to elucidate the reasons for the widespread dissemination of the virus.

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